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AN 'IN VIVO' MODEL FOR THE DEFINITIVE ANALYSIS OF CELLULAR INFLAMMATION IN NORMAL AND IRRADIATED RATS

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13. ABSTRACT

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# AN IN VIVO MODEL FOR THE DEFINITIVE ANALYSIS OF CELLULAR INFLAMMATION IN NORMAL AND IRRADIATED RATS

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## FOREWORD
(Nontechnical summary)

At an inflammatory site an increasing number of white blood cells leave the blood and move into the surrounding tissue to fight infection. Their success depends on the number of cells capable of entering the inflammatory area, their functional capability and the degree of infection. It is important to measure these parameters as precisely as possible. To this end, a plastic cup designed by the authors was surgically inserted under the skin of normal and irradiated rats. The cup collected the white blood cells which moved into the inflamed tissue. An accurate assessment of cellular movement was possible by measuring the number and type of white blood cells in the cup at various time intervals following implantation. These data will be useful in the design of therapeutic measures for increased efficiency in treating infection, particularly after exposure to ionizing radiation.

## ABSTRACT

A new *in vivo* model which gives a more accurate picture of cellular inflammation was developed. It involves a subcutaneously implanted plastic cup which can easily be used to assess quantitatively dynamic exudate cell migration. The use of this model in normal and irradiated rats has provided new insights into the kinetics of exudate cells during acute inflammation. Under the conditions described in this study, it appears that mononuclear cells are the dominant type cells in inflammatory exudates much earlier than has been previously reported in studies using less quantitative models.

## I. INTRODUCTION

Polymorphonuclear cells are the first to appear in high concentrations in an inflammatory exudate.[6,14,16,17] At first, mononuclear cells constitute a small fraction of the leukocytic population at the inflammatory site; but, they increase in number with time and usually have replaced the polymorphonuclear cell as the predominant cell type within 24 hours.[6,14,16,17]

Many in vivo models and techniques have been developed to study this sequence of cellular events[1-15,18] but with questionable results. For example, the cover slip technique of Rebuck and Crowley[8] is used extensively but, similar to others,[14,17] it is only semiquantitative. The cantharidin-induced skin blister used by Boggs et al., although quantitative, is limited to the study of granulocyte migration in humans.[2] Lundgren and Lindhe's subcutaneously implanted cylinders used for studying aseptic exudates while apparently permitting a quantitative analysis show results that differ from those of other studies in terms of cellular appearance and concentration.[5] One technique which is both quantitative and qualitative is the site analysis of Paz and Spector.[6] It is suitable for use in animals and for the analysis of all types of cellular reactions but is cumbersome because it involves the histological preparation and sectioning of skin specimens and may not represent a dynamic condition.

This report describes the development and utilization of an in vivo model which simulates more closely cellular inflammation. It involves a subcutaneously implanted plastic cup which can easily be used to assess exudate cell migration quantitatively. The exudate cellular profile produced by this model shows the usual early polymorphonuclear cell predominance followed by a later mononuclear predominance. Further,

it reveals a unique quantitative cellular response to inflammation which is a measure of dynamic exudate cell migration within inflamed tissue.

## II. METHODS

Animals. Four hundred and sixty-eight young mature male Sprague-Dawley albino rats, weighing 250 $\pm$ 20 g, from the AFRRI colony were caged individually, room temperature 22$^{\circ}$C, and given food and water *ad libitum*.

Cup design. The cup is made from acrylic, has a 15-mm diameter, is 5 mm high and has a 0.2-ml capacity (Figure 1).

Cup implantation. Each rat was anesthetized with a 36 percent solution of chloral hydrate injected I.P. (10 ml/kg body weight). Its back was shaved, scrubbed with pHisoHex, and washed with a 70 percent solution of ethyl alcohol. A 3-cm longitudinal, paravertebral, full-thickness skin incision was made. A subcutaneous pouch

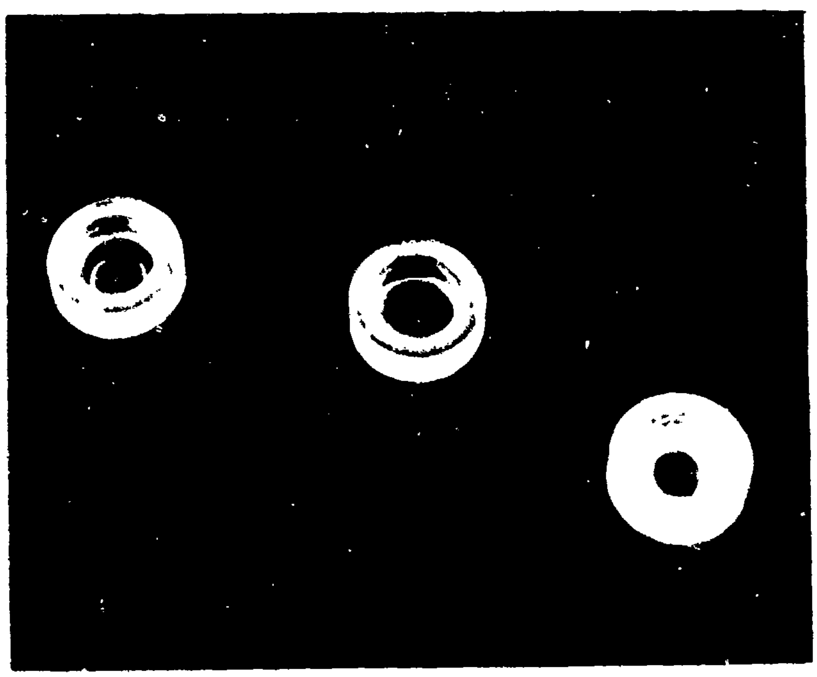

Figure 1. Acrylic cup

was produced by a blunt dissection, into which a round acrylic cup filled with bacteria-free Hanks' balanced salt solution was inserted 2 cm from the skin incision. The incision was then closed with a stainless steel wound clip.

Control group. In this study a cup and adjacent to it a 15-mm round acrylic cover slip were implanted subcutaneously in each of 156 rats. The subcutaneous cups and the cover slips were removed from a group of 12 rats at each of 13 sampling times from 5 to 75 hours following implantation.

The exudate in each cup was aspirated with a syringe, and the volume measured. A smear of the exudate fluid from each cup was made for differential cell counting. The acrylic cover slips were stained and examined in the same manner. An aliquot from each of the cups was also taken to determine the cell count per cubic millimeter of exudate using a Coulter Model F cell counter. Using the values determined above, the total number of the various exudate cell types recovered from each cup was calculated by using the following:

$$\text{WBC's} \times 10^3 \times \text{EV} \times \text{FLCT} = \text{total number of cell type in cup}$$

where WBC = number of cells per cubic millimeter of exudate (Coulter count); EV = volume of exudate fluid in the cup (milliliters); and FLCT = fraction of leukocyte cell type. The mean number of cells per cup for each cell type was calculated and plotted on a graph for each of the sampling times.

Exudate samples were also taken from 12 animals 3 hours following cup implantation. However, there were so few cells present in any one sample that all 12 samples had to be pooled and concentrated by centrifugation before they could be prepared for differential determinations.

Irradiated group. In this study a cup was implanted subcutaneously in each of 312 rats. The sampling procedure was the same as that used in the study with control rats. However, 2 hours prior to implantation they were divided into four groups, three of which were subjected to ionizing radiation from the AFRRI $^{60}$Co source. The first group received 250 rads, the second group 500 rads, and the third group 750 rads measured as midline doses. The fourth group was a nonirradiated control group. The midline dose rate for all exposures was 20 rads/minute.

Histology. A 45-mm square full-thickness skin specimen immediately above the inserted cups in five irradiated rats was excised 24 hours following implantation. Longitudinal sections of the skin lying in contact with the upper liquid surface of the Hanks' balanced salt solution were cut at 6 μm and stained with hematoxylin and eosin and examined for evidence of inflammation. These sections were compared with normal rat skins prepared in the same manner.

Statistics. Differences between experimental means were tested with the Student's "t" test.

## III. RESULTS

The results of the first study using nonirradiated rats are presented in Figure 2. Mononuclear cells (monocytes and macrophages) were the dominant cell type recovered from the indwelling cups at all sampling times from 5 to 75 hours following implantation. The mean number of mononuclear cells per cup increased slowly up to 16 hours, then rose rapidly peaking at approximately $45 \times 10^6$ cells per cup at 30 hours. A cellular decrease followed ending at 45 hours, and vacillated thereafter until the last sampling time at 75 hours. The absolute number of granulocytes

which appeared in the cups increased up to 35 hours, then gradually declined. Lymphocyte numbers in the samples remained constantly low throughout the study, never exceeding 250 x $10^3$ cells per cup. The volume of exudate fluid collected from each cup ranged from 0.15 ml to 0.20 ml.

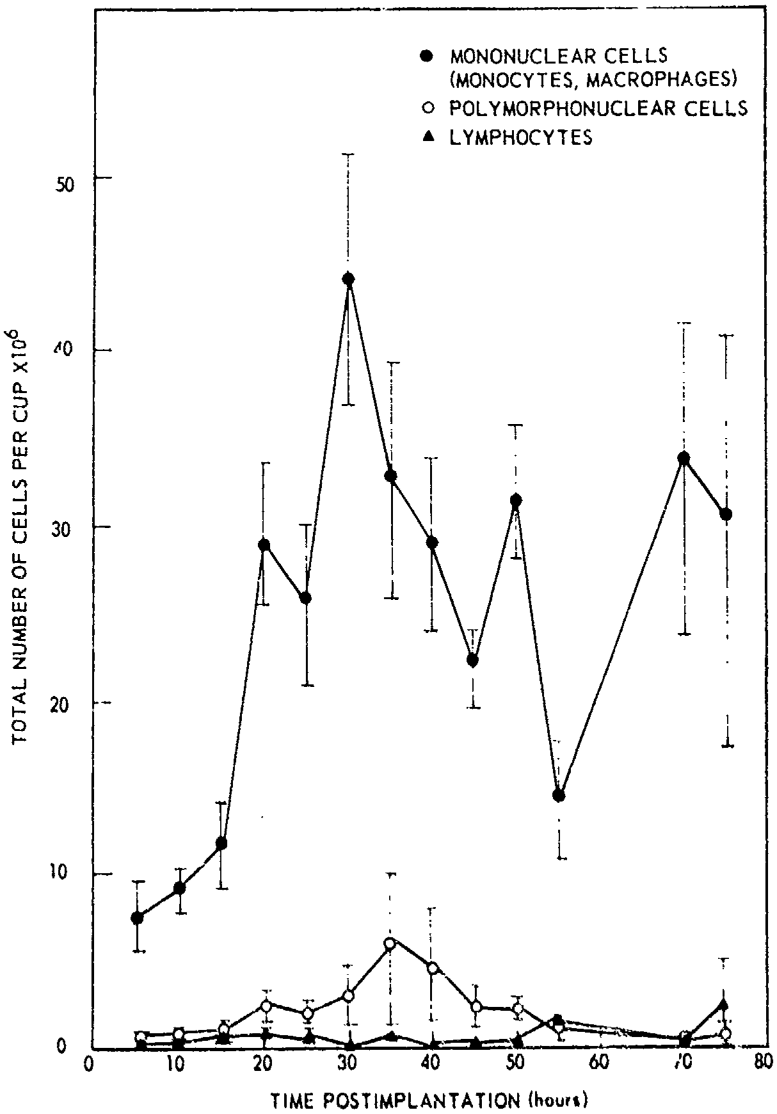


Figure 2. Inflammatory cellular response in rats following cup implantation

The mononuclear cells in irradiated animals were significantly reduced ($p<0.001$) from nonirradiated control values for all sampling times from 20 to 75 hours (Figure 3).

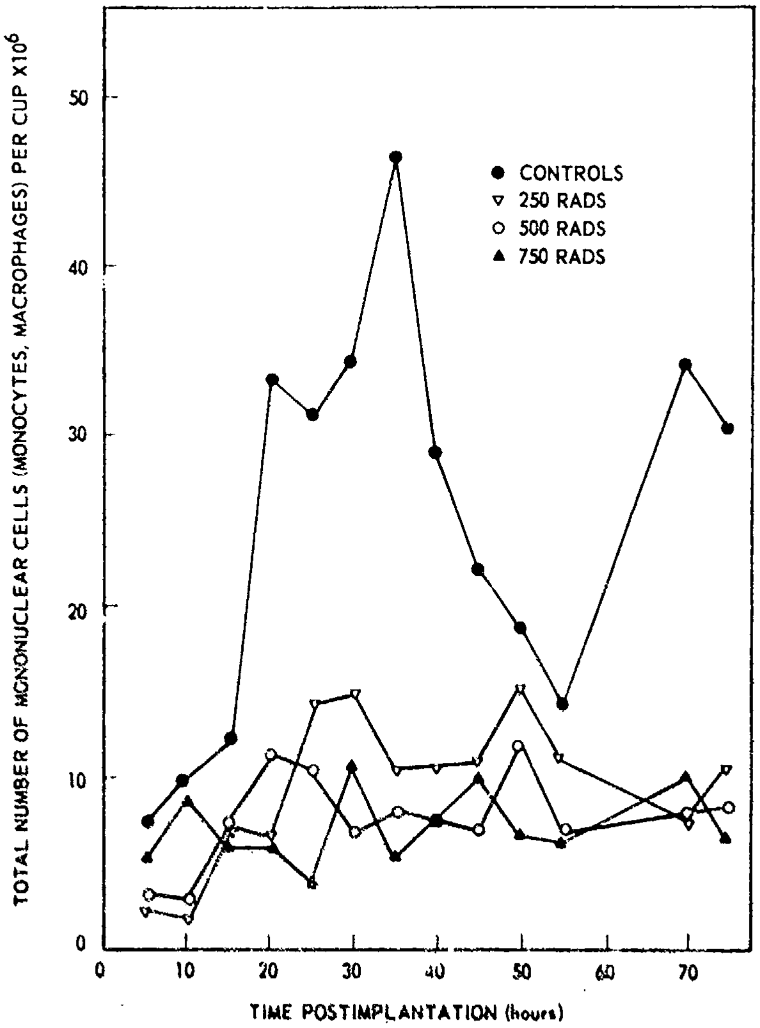


Figure 3. Inflammatory mononuclear cell response following cup implantation in rats exposed to 250, 500 or 750 rads of gamma radiation

Granulocyte numbers were below control values at all sampling times for rats that received either 250 or 500 rads. However, granulocyte values for the 750-rad

group exceeded control values on the 1st and 3rd day (Table I). Lymphocytes were drastically reduced in many samples and totally absent from others for all doses of radiation (Table I).

Table I. Granulocyte and Lymphocyte Response (mean number per cup) in Rats Following Cup Implantation

| | Rads | Time postimplantation (hours) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 70 | 75 |
| Granulocytes | 0 | 47.74 | 46.97 | 101.86 | 467.65 | 292.16 | 402.35 | 1043.38 | 390.68 | 179.03 | 195.14 | 76.54 | 37.69 | 52.16 |
| | 250 | 10.36 | 7.59 | 11.65 | 24.60 | 51.48 | 173.80 | 18.26 | 27.38 | 60.72 | 63.25 | 41.14 | 18.26 | 44.66 |
| | 500 | 28.93 | 19.47 | 69.76 | 10.92 | 55.33 | 20.13 | 177.87 | 105.70 | 123.51 | 193.27 | 73.70 | 22.76 | 9.68 |
| | 750 | 118.47 | 66.14 | 202.75 | 274.23 | 570.02 | 264.55 | 218.35 | 333.76 | 535.04 | 640.97 | 309.88 | 225.17 | 86.68 |
| Lymphocytes | 0 | 28.63 | 19.44 | 50.20 | 20.46 | 23.53 | 3.22 | 20.11 | 0.0 | 4.57 | 13.76 | 25.96 | 46.30 | 64.49 |
| | 250 | 0.18 | 0.88 | 0.0 | 3.08 | 16.50 | 1.45 | 0.88 | 0.0 | 0.0 | 0.0 | 0.0 | 11.22 | 0.0 |
| | 500 | 3.63 | 0.0 | .44 | 1.54 | 10.89 | 0.0 | 2.86 | 2.35 | 3.85 | 1.10 | 0.33 | 37.95 | 28.05 |
| | 750 | 5.61 | 2.53 | 2.61 | 1.65 | 12.54 | 3.08 | 2.75 | 0.0 | 0.0 | 4.84 | 0.0 | 37.93 | 0.88 |

Skin specimens taken from the site above the subcutaneous cups showed marked evidence of inflammation. The vasculature was congested and engorged with blood. In many of the smaller veins, venules and capillaries there was a pavementing of the endothelium with polymorphonuclear leukocytes (PMN's) and monocytes. In the perivascular connective tissue adjacent to these vessels many PMN's and mononuclear cells as well as fibrils were present in the proteinaceous exudate. A few lymphocytes were also present. Vasculitis was apparent in many veins and venules and the walls of some of the small arterioles and arteries in the vicinity of the inflammation were edematous.

The majority of the granulocytes recovered from the cups were segmented, although a few band forms could be identified. The mononuclear cells regularly followed a pattern of first appearing as cells much like monocytes, then as nonvacuolated macrophagic mononuclear cells with a round nucleus. Later vacuolated

mononuclear cells and a few multinucleated giant cells could be seen in most samples after 35 hours.

Sixty-five to seventy percent of the cells on the subcutaneously implanted acrylic cover slips were monocytes and macrophages at all sampling times. Between 25 and 30 percent were polymorphonuclear cells and less than 1 percent were lymphocytes. Differential counts made from the pooled and concentrated exudate at 3 hours showed that 75 percent of the cells present were polymorphonuclear, approximately 25 percent were monocytes and macrophages and less than 1 percent were lymphocytes.

## IV. DISCUSSION

A new *in vivo* model which can measure dynamic exudate cell migration during acute inflammation is presented. In many respects this model represents an improvement over other models which are currently being used. For instance, many of the models lack simplicity and facility. The site analysis technique of Paz and Spector[6] is cumbersome because it requires the histological preparation and sectioning of skin specimens. Furthermore, it does not provide a site from which exudate can be collected. Other models which do provide a site for exudate collection such as the peritoneal cavity,[13] the subcutaneous air pocket[10,11,18] and the cantharidin-induced blister,[2] do not have a collection site with a fixed volume and are difficult to quantitate. The indwelling plastic cup described herein is not only a collection site with a fixed volume, but a site from which exudate cells can be collected and into which cells and other substances can easily be injected.

The exudate cellular profile produced by this model closely simulates the cellular response which occurs following most types of injury.[6,14,16,17] Mononuclear cells

predominate after 5 hours (Figure 2) and measurements made at 3 hours indicated an early infiltration of PMN's. A number of other quantitative models fail to reflect this picture. For instance, Zurier et al. recently reported that the majority of exudate cells which migrate into an aseptic inflammatory focus within the first 27 hours were PMN's.[18] Lundgren and Lindhe reported that PMN's were the predominant cell type found in the aseptic exudate recovered from subcutaneous perforated cylinders up to 4 days following implantation.[5] Why these quantitative models fail to show tne usual early mononuclear cell predominance is not clear.

The exudate cellular profile in the present study also reveals that the number of moronuclear cells vacillate considerably from one sampling time to another (Figures 2 and 3). Such pronounced vacillations have not previously been reported probably because most models only sample a small portion of inflamed tissue. In this study all of the histological sections made from the skin above the cup showed evidence of inflammation. This suggests that the exudate cells which migrated into the cup probably came from all regions within the tissue.

This model is also unique because it is one of the few ever developed which is fully quantitative. Most of the reports in the literature are based on information derived from models which can only qualitatively depict exudate cell migration. Discrepancies between the results derived qualitatively and those derived quantitatively have been described by Southam and Levin.[12] Such discrepancies were also evident during this study. Figure 2 shows the quantitative cellular profile produced when this model is used, and it can be seen that the macrophages always represented more than 80 percent of the total number of cells present at all sampling times. However,

differential white blood cell counts made from acrylic cover slips subcutaneously implanted in these same animals revealed a different qualitative picture. They indicated that the number of macrophages deposited on the cover slips never exceeded more than 70 percent of the cells. A similar discrepancy existed between the results in the radiation study (Figure 3) and those reported by Volkman and Gowans.[15] They found that while macrophages were abundant on subcutaneously implanted cover slips in rats after receiving 400 rads of x radiation, they were virtually absent from animals that received 750 rads. Yet, in this study, no significant difference was found between the number of macrophages recovered from rats exposed to 750 rads and those exposed to 250 or 500 rads of gamma radiation. This suggests that models which are not fully quantitative may not have the ability to detect low concentrations of exudate cells.

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